United States Department of Agriculture,

BUREAU OF CHEMISTRY—Circular No. 76.

H. W. WILEY, Chief of Bureau.

DETERMINATION OF MALIC ACID.

By P. B. DUNBAR and R. F. BACON, *Assistant Chemists, Division of Foods.*

INTRODUCTION.

The method described herein is based on the fact first observed by Walden [1] that under certain conditions uranium salts produced a very marked increase in the specific rotation of *l*-malic acid. Walden noticed that a similar but less marked effect is produced by uranium salts on *d*-tartaric acid, *d*-methyl tartrate, *l*-quinic, and *l*-mandelic acids, the increased rotation being in the same direction as the rotation of the aqueous solution of the substance. He concluded that the effect is limited to the active oxy-acids. As stated by Walden, the requisites of an agent which is to be used for the detection of malic acid by optical means are: (1) That the direction of rotation shall be constant, and (2) that the increase in rotation shall be as large as possible. This, of course, applies also to the quantitative estimation of malic acid. In addition, the agent in question should have little or no influence on any other substances which may occur in conjunction with malic acid. It has long been known that certain substances such as the oxygen compounds of boron [2] arsenic, antimony, molybdenum, and tungsten [3] have the power of increasing the specific rotation of the oxy-acids. Uranium salts, however, are the only ones which fulfill the requirements just stated with any degree of satisfaction.

The authors have recently had the privilege of reading an article by P. A. Yoder entitled "A Polariscopic Method for the Determination of Malic Acid and Its Application in Cane and Maple Products." [4] This paper was presented at the forty-second meeting of

---

[1] Ber. d. chem. Ges., 1897, **30** (3) : 2889.
[2] Biot, Mém. de l'acad. roy. sci., 1838, **16**: 229 ; Ann. chim. phys., 1884 (3), **11**: 82 ; 1850, **29**: 341, 430 ; 1860, **59**: 229. Pasteur, Ann. chim. phys., 1860 (3), **59**: 243.
[3] Gernez, thr. Landolt's Optical Rotation of Organic Substances, tr. by Long, p. 248.
[4] J. Ind. Eng. Chem., 1911, **3**: 563.

3123°—Cir. 76—11

the American Chemical Society in July, 1910, and contains the results of a large amount of work. The method outlined depends on the use of a uranium salt, but in other respects it differs materially from the one here proposed. With this exception, no one appears to have attempted the quantitative determination of malic acid by treatment with uranium.

THE METHOD IN BRIEF.

The method in brief consists in treating a portion of the neutralized[1] solution containing malic acid with uranyl acetate and polarizing it. The algebraic difference between the reading so obtained and that of the untreated solution is multiplied by the factor 0.036 to obtain the percentage of malic acid present. With the exception of d-tartaric acid, none of the common optically active substances interfere with the determination, and consequently they need not be removed. Uranyl acetate slightly decreases the rotation of sugars. This may give rise to an error in the estimation of small amounts of malic acid in the presence of large amounts of invert sugar. Hence, when the rotation of the original solution is negative and the approximate amount of sugar or malic acid is unknown, or when more than 10 per cent of reducing sugars and less than 0.25 per cent of malic acid are present, it is necessary to precipitate the malic acid and treat the filtrate with uranyl acetate also. In this case the polarization of this filtrate, instead of that of the untreated solution, is subtracted from the polarization of the solution containing both malic acid and uranyl acetate, and the difference is multiplied by 0.036.

DETAILS OF THE METHOD.

Dilute a measured volume of the solution, usually 10 cc, with quite a large volume of water, add phenolphthalein, and titrate with standard alkali to a decided pink color. Transfer another measured portion of the solution (75 cc is a convenient volume) to a 100 cc graduated flask, and add enough standard alkali, calculated from the above titration, to neutralize the acidity. A slight excess of alkali is not objectionable. If the solution is dark colored, add 5 or 10 cc of alumina cream. Dilute to the mark, mix thoroughly, and filter if necessary through a folded filter.

(1) Treat about 25 cc of the filtrate with powered uranyl acetate, adding enough of the salt so that a small amount remains undissolved after two hours. Two and one-half grams of uranyl acetate will usually be sufficient, except in the presence of large amounts of malic acid. In case all the uranium salt dissolves more should be added. Allow the mixture to stand for two hours, shaking fre-

---

[1] If no mineral acids are present it is not necessary to neutralize the malic acid.

quently. Filter through a folded filter until clear and polarize if possible in a 200 mm tube. If the solution is too dark to read in a 200 mm tube, a 100 or 50 mm tube may be used. It is desirable, however, to use the longest tube possible in order to obtain the maximum rotation. This solution and reading will hereafter be designated as (1).

(2) Treat the remainder of the original filtrate with powdered normal lead acetate until no further precipitation results. Cool in an ice bath and filter through a folded filter until clear. Warm the filtrate to room temperature and add a small crystal of lead acetate to determine whether the precipitation is complete. If no further precipitate results, remove the excess of lead completely with anhydrous sodium sulphate, filter until clear, and polarize. Designate this filtrate as Solution (2) and its polarization as Reading (2). Care should be taken to add no more lead acetate to the solution than is necessary for complete precipitation, as lead malate is soluble in an excess of lead acetate. Solutions which are sufficiently clear and contain less than 10 per cent of sugar may be polarized directly without treatment with lead acetate.

(3) If Reading (2) is negative treat a portion of Solution (2) with uranyl acetate in the manner described under (1) and polarize. Designate this as (3). If Reading (2) is positive, Reading (3) need not be made.

Polarize at room temperature with white light, taking care that all solutions are polarized at the same temperature. Make at least six readings in each case and take an average of these. In this work a standard, Lippich type, triple field saccharimeter was used, the light being furnished by an electric bulb placed behind a ground-glass plate. Calculate all readings to the basis of a 200 mm tube. If Reading (3) is numerically less than Reading (2), the latter should be discarded; otherwise use Reading (2) in the subsequent calculation. Multiply the algebraic difference between this reading and Reading (1) by 0.036. The product will equal the percentage of malic acid $\left\{ \begin{array}{l} CH_2COOH \\ | \\ CHOHCOOH \end{array} \right\}$ in the solution as polarized.

EFFECT OF URANIUM SALTS.

The method just described was developed in the course of some work on the composition of the apple. If uranium salts produce an increase in the rotation of malic acid only, it would theoretically be possible to estimate the amount of malic acid in a solution containing sugars and other organic compounds by simply polarizing

the solution before and after the addition of the uranium salt and multiplying the difference in the readings by a factor determined experimentally. As was previously stated, the rotation of d-tartaric acid is also increased by uranium salts. Consequently, this method can not, without modification, be applied to a solution containing this acid. We have determined that, as would be expected, inactive lactic, succinic, and citric acids are unaffected by salts of uranium. There are, therefore, no serious difficulties in the way of applying this method to the determination of malic acid in the absence of d-tartaric acid. Work is now in progress on the estimation of tartaric acid by a similar method.

In the earlier experiments of this investigation an attempt was made to use uranyl nitrate and potassium hydroxid as reagents, as recommended by Walden. This author states that a maximum rotation is obtained by the use of 4 molecules of potassium hydroxid and 1 to 4 molecules of uranyl nitrate $[UO_2(NO_3)_2+6H_2O]$ for each molecule of malic acid. He prefers the nitrate to the acetate because of the greater solubility of the former salt.

Uranyl nitrate, however, has one serious disadvantage. Its solution in water always contains nitric acid due to hydrolysis. It is impossible to obtain the maximum polarization in the presence of free mineral acid and it is therefore necessary, as Walden pointed out, to neutralize the solution when uranyl nitrate is used. The color of the solution always makes this operation very difficult. Many attempts were made to use uranyl nitrate, but with indifferent results. After addition of the uranyl nitrate solution to that containing malic acid, a solution of potassium hydroxid was added drop by drop until a precipitate just began to form. This was dissolved either by addition of a drop of nitric acid or of uranyl nitrate solution. The results, however, were very irregular. In some cases the theoretical recovery of malic acid was obtained, while in others the results were very much too low. Attempts to neutralize the solution, using various indicators, were made with similar results. No better success was attained when the amount of alkali required to neutralize both the uranyl nitrate and the malic acid solution was determined in advance and the calculated amount added to the mixture.

Uranyl acetate was then substituted for the nitrate in the hope that the presence of a weak organic acid like acetic acid would not influence the rotation of the uranium-malic complex. As a further precaution the solution to be examined was carefully neutralized before the addition of the uranium salt. The concentration of H ions in a solution containing acetic acid and sodium acetate is so slight that it was believed its influence on the rotation would be

negligible. This procedure proved satisfactory and no further diffi-
culty was experienced in this direction.

A number of experiments were made to determine what effect, if
any, is produced by an excessive amount of uranyl acetate. The re-
sults all showed that such an excess does not influence the rotation of
the solution. On the other hand, it is quite possible to add too small
an amount of the salt to the solution under examination. In the case
of solutions containing a comparatively large amount of malic acid,
several hours may elapse before a maximum change in rotation is ob-
tained. To test this point a solution containing 2.94 per cent of malic
acid as sodium malate was treated with an excess of uranyl acetate,
shaken frequently and polarized at intervals. The polarizations ob-
tained were as follows:

	°V
After 10 minutes	−60. 4
35 minutes	−68. 7
1 hour	−74. 1
2 hours, 10 minutes	−82. 0
2 hours, 45 minutes	−82. 8
4 hours, 10 minutes	−83. 0

This indicates that after two hours the reading is practically con-
stant, since a difference of 1° corresponds to only 0.036 per cent of
malic acid. It must be remembered, however, that frequent agitation
is also needed if a maximum is to be attained in this time.

An approximate determination of the solubility of uranyl acetate
at ordinary temperatures gave the following results:

	Grams per 100 cc.
1. In water	8. 5
2. In a solution containing 1.25 per cent of malic acid as sodium malate	11. 96
3. In a solution containing 2.5 per cent of malic acid as sodium malate	13. 32

REMOVAL OF THE MALIC ACID.

Walden reports $[a]_D$ for a 0.65 per cent solution of l-malic acid
as approximately −0.77 circular degrees, as compared with −475°
(circular) for the uranium complex. It is evident, therefore, that in
ordinary concentrations the rotation of free malic acid is negligible.
However, as has been said, it is sometimes necessary to remove the
malic acid from the solution before making reading (2) for the reason
that the addition of uranyl acetate causes a decrease in the rotation of
strong sugar solutions.[1] To determine the extent of this effect, 13
grams of uranyl acetate were added to 100 cc of various sugar solu-
tions and allowed to stand one hour with frequent shaking. The so-

[1] Rembach and Weber (Zts. physikal. Chem., 1905, 51:491) have noticed that uranyl
nitrate produces a slight increase in the specific rotation of solutions of dextrose and
levulose containing 7.2 grams of the sugar in 100 cc of solution.

lutions were then polarized in 200 mm tubes with white light and the readings compared with those of the untreated solution. After standing overnight the readings were repeated.

TABLE I.—*Effect of uranyl acetate on the rotation of sugars.*

Sugar.	Grams in 100 cc.	Sugar solution, after 1 hour.		Sugar solution, after standing over night.	
		Without uranium salt.	After addition of uranium salt.	Without uranium salt.	After addition of uranium salt.
		$°V.$	$°V.$	$°V.$	$°V.$
Cane sugar	26	+100.0	+ 97.5	+100.0	+ 97.3
Levulose	26	−125.4	−120.8	−126.0	−121.2
Dextrose	26	+ 82.4	+ 78.8	+ 80.4	+ 78.8
Invert sugar	50	− 61.9	− 60.0	....	....
Do	25	− 28.6	− 27.0	....	....
Do	10	− 10.9	− 9.7	....	....

The results show a decided reduction in rotation which would have a serious effect on the determination of small amounts of malic acid in the presence of large amounts of sugar. To avoid inaccuracy from this cause it is necessary in some cases to remove the malic acid as completely as possible from solution (2) and then to add uranyl acetate to the filtrate. For reasons which will be given later this procedure is never followed when the rotation of solution (2) is positive. Normal lead acetate is used to precipitate malic acid. Even when the removal of the malic acid is not necessary it is advisable to clarify the solution with lead acetate and then remove the excess of lead in order to obtain an easily readable solution. In the earlier work a solution of lead acetate was added to the malic-acid solution before diluting to the mark. It was found, however, that the volume of the precipitate in some cases seriously changed the concentration of the solution, especially where sugar solutions inverted with hydrochloric acid were used, and the solid normal lead acetate, $Pb(C_2H_3O_2)_2 + 3H_2O$, was therefore substituted. This salt contains 4.7 per cent of water of crystallization and introduces a negligible dilution error in the amounts used. An attempt was made to use Horne's dry lead subacetate, but the results were not satisfactory.

Dry neutral potassium oxalate was at first used to remove the excess of lead from the solution. It was later found that the rotation of sugar solutions is affected to some extent when this salt is added to an invert sugar solution containing lead acetate. An approximately 25 per cent solution of invert sugar polarized −29° before and after addition of lead acetate. After precipitating the lead with potassium oxalate, however, the filterate polarized −29.6°. Conse-

quently anhydrous sodium sulphate was substituted for potassium oxalate with satisfactory results.

Lead malate is somewhat soluble in water, lead acetate, and sugar solutions. This fact has caused difficulty at two points during the development of the method. It was early discovered that when a solution of sodium malate is treated with lead acetate the filtrate has a positive polarization amounting to as much as one degree. Previous to this discovery it was not thought necessary to remove the lead from solution (2) before polarization. A more serious difficulty was encountered when it was found necessary to treat solution (2) with uranyl acetate. The presence of even a slight amount of malic acid in this solution after removal of the lead will of course cause a change in rotation when the uranium salt is added. To obtain the best possible separation of lead malate, therefore, the solution is cooled in an ice bath before filtering. This procedure is sufficient to remove most of the malic acid when quantities less than 0.2 per cent are present, but if large amounts of the acid are present a very considerable error may be introduced by the change in rotation caused by the formation of the uranium-malic complex. This is illustrated by the following experiment:

Four solutions containing known amounts of malic acid, as sodium malate, were treated with lead acetate at room temperature, the lead removed with potassium oxalate, and the filtrate treated with uranyl acetate for one-half hour, with frequent shaking. The filtrates were polarized with the following results:

Per cent of malic acid:	°V.
0. 099	— 0. 1
. 199	— 1. 1
. 99	— 3. 2
1. 99	—15. 5

When the solution, which has been treated with lead acetate, is cooled in ice water before filtering. the rotation with uranyl acetate is much decreased, so that if small amounts of malic acid occur in the presence of large amounts of invert sugar, Reading (3) is less than Reading (2). When comparatively large amounts of malic acid are present. however, Reading (3) is likely to be greater than (2), and for this reason both readings are made when the approximate amount of invert sugar or malic acid are unknown, and the smaller is used in calculating the percentage of malic acid present.

In the presence of large amounts of sucrose the error due to the effect of the uranium salt on sugar is in the same direction as that due to the presence of traces of malic acid. This will be clearly seen from the following determinations on an approximately normal solution of sucrose containing 0. 24 per cent of malic acid.

TABLE II.—*Determinations on a normal solution of sucrose containing 0.24 per cent of malic acid.*

Readings.	Polarization.	Difference.	Malic acid found.	Recovery.
	° V.	° V.	Per cent.	Per cent.
1...	+88.5	....	....	....
2...	+95.6	[1] 7.1	0.26	108.3
3...	+93.8	[2] 5.3	.19	79.2

[1] Difference between (1) and (2).   [2] Difference between (1) and (3).

It will be seen that when the difference between readings (1) and (2) is used in the calculations a somewhat high recovery is obtained. This is to be expected since the decrease in rotation due to the effect of the uranium salt on sugar is added to that due to the rotation of the uranium malic complex. Consequently, Reading (1) is lower, and the difference between (1) and (2) is higher, than it should be. Reading (3) is decreased by the combined effect of the action of the uranium salt on sugar and also on the traces of malic acid present. Hence, the difference between (1) and (3) is too small. The percentage recovery obtained by using the difference between readings (1) and (2) is not excessively high and this is the largest error that would be obtained due to the action of uranium salts on sucrose, assuming that no more concentrated solution than one of normal sugar is examined. Therefore, while not theoretically correct, it is practically permissible to dispense with Reading (3) entirely when working with solutions in which Reading (2) is positive. It would, of course, be possible to avoid error from this source by inverting the solution before beginning the determination, then neutralizing, and diluting to a definite volume. This has not been found to be necessary in practice, however. When it is known that the solution under examination contains either less than 10 per cent of invert sugar or more than 0.25 per cent of malic acid it is unnecessary to make Reading (3).

Solutions are frequently encountered which are too dark to polarize without clarification. It was found that alumina cream can be used for this purpose without affecting the results.[1]

The question arises as to whether uranium salts produce the same effect on levo, dextro, and inactive malic acid. For ordinary work on natural products this question is of little importance, since, as far as the literature goes, malic acid appears to occur in nature only in the levo form. W. Gintl[2] reports the presence of the inactive form in the leaves of *Fraxinus excelsior* L., but this discovery does not appear to have been confirmed since that time.

[1] With very dark solutions such as fruit juices, C. W. Clark, of this laboratory, has found that a few drops of bromin may often be used to decolorize without affecting the results.

[2] Jahresber. Chem., 1868, p. 800.

It is well known that the specific rotation of aqueous solutions of *l*-malic acid varies greatly with concentration,[1] changing from minus to plus in the higher concentrations. The addition of concentrated sulphuric acid[2] will also cause a change in rotation. Some experiments were made to throw light on the question as to whether such differences in the specific rotation of *l*-malic acid affect the rotation of the uranium malic complex. A solution containing 13.95 grams of *l*-malic acid in 28 cc of solution polarized +1.1°. This solution was diluted and the malic acid determined by the uranium method, the theoretical recovery being obtained. This same solution was treated with concentrated sulphuric acid and after standing over night polarized +2.7. After standing four days the rotation was found to be the same. On dilution the theoretical recovery was again obtained by the uranium method.

DETERMINATION OF THE FACTOR.

A large number of experiments were made to determine the factor for calculating the percentage of malic acid from the polarization. Neutral solutions containing known amounts of malic acid were treated with uranyl acetate, polarizing in a 200 mm tube with white light and dividing the percentage of malic acid present by the reading in degrees Ventzke. In some cases this determination was made in the presence of invert sugar. The details of the experiment are given in Table IV, page 10. It will be observed that there is a gradual decrease in the factor with increase in concentration of malic acid. The increase is not sufficient, however, to seriously affect results in the ordinary concentrations found in natural products. The average of 27 determinations gave a factor 0.036, and this was accordingly adopted for use in all calculations. A number of determinations in which sodium light was used instead of white light showed a slightly smaller specific rotation with the former.

TABLE III.—*Specific rotation in circular degrees (10 cm tube).*

Per cent malic acid.	$[a]_J$.	$[a]_D$.
0.098	−442	−436
.294	−457	−448
.489	−470	−464
.978	−476	−470
1.956	−495	−488

With very clear solutions containing large amounts of uranium-malic complex difficulty may be experienced in obtaining the neutral point on account of the decided blue color developed in one segment of the field. This can be eliminated to a great extent by the use of a 3 cm cell containing a 3 per cent solution of potassium bichromate.

[1] Schneider, Ann. Chem. (Liebig.) 1881, **207**: 262.   [2] Ibid., p. 279.

The use of such a screen has the additional advantage of lowering the specific rotation of the uranium-malic complex somewhat and consequently partially correcting for the normal rise in specific rotation due to concentration.

TABLE IV.—*Determination of factor.*

Number of determination.	Invert sugar present (approximate).	Malic acid present.	Polarization.		Difference between Readings (1) and (2).	Factor calculated.	Malic acid found, using factor 0.036.	Recovery.
			Solution 1—with uranium acetate.	Solution 2—without uranium acetate.				
	Per cent.	Per cent.	° V.	° V.			Per cent.	Per cent.
1	....	2.48	−74.6	....	74.6	0.033	2.69	108.5
2	....	1.49	−43.5	....	43.5	.034	1.57	105.4
3	2.5	1.49	−46.8	−3.0	43.8	.034	1.58	106.0
4	....	.99	−28.2	....	28.2	.035	1.02	103.0
5	....	.99	−28.5	....	28.5	.035	1.03	104.0
6	2.5	.99	−31.4	−2.9	28.5	.035	1.03	104.0
7	5.0	.99	−34.2	−5.9	28.3	.035	1.02	103.0
8	....	.99	−29.0	....	29.0	.034	1.05	106.1
9	....	.80	−22.8	....	22.8	.035	.82	102.5
10	....	.60	−16.8	....	16.8	.036	.61	101.7
11	....	.50	−14.2	....	14.2	.035	.51	102.0
12	....	.50	−14.2	....	14.2	.035	.51	102.0
13	2.5	.50	−17.0	−2.8	14.2	.035	.52	104.0
14	5.0	.50	−19.8	−5.9	13.9	.036	.50	100.0
15	5.0	.50	−19.7	−6.0	13.7	.036	.50	100.0
16	....	.40	−11.3	....	11.3	.035	.41	102.5
17	....	.30	− 8.5	....	8.5	.035	.31	103.3
18	....	.25	− 7.0	....	7.0	.036	.25	100.0
19	2.5	.25	− 9.9	−2.9	7.0	.036	.25	100.0
20	5.0	.25	−12.5	−5.8	6.7	.037	.24	96.0
21	....	.20	− 5.7	....	5.7	.035	.21	105.0
22	....	.10	− 2.8	....	2.8	.036	.10	100.0
23	2.5	.10	− 5.6	−2.8	2.8	.036	.10	100.0
24	5.0	.10	− 8.5	−5.8	2.7	.035	.10	100.0
25	....	.050	− 1.3	....	1.3	.038	.047	94.0
26	....	.050	− 1.3	....	1.3	.038	.047	94.0
27	2.5	.050	− 4.0	−2.75	1.25	.040	.045	90.0
Average factor.	....	....	....	....	....	.036	....	....

DISCUSSION OF RESULTS.

The results which have been obtained in the application of the method are tabulated in Tables V and VI, pages 11 and 12, which are in the main self-explanatory. Table V contains the results of determinations in which known amounts of malic acid were added by the analyst, while the determinations recorded in Table VI were made on solutions in which the amount of malic acid was unknown. The percentages of invert sugar given in the second column of Table V are only approximate. This accounts for the fact that in some cases solutions containing the same amount of sugar and malic acid show a larger difference in polarization than can be accounted for by errors in reading. Stock solutions containing 5 or 10 per cent of carefully weighed C. P. malic acid were made up and the percentages of acid present were determined by titration against standard alkali. A measured portion of the stock solution was then added to a definite volume of invert-sugar solution and the mixture diluted

to the desired concentration. The sugar solutions always contained more or less sodium chlorid resulting from the neutralization of the acid used for inversion. New malic acid stock solutions were made up every two or three days, as a growth of mold usually appears after a few days' standing. In many cases Reading (3) was not made. Wherever possible it is given, however. It will be noted that in many cases Reading (3) is higher than (2) for the reason previously explained. When the former is lower, the percentage of malic acid is calculated from it. The most favorable concentrations of malic acid appear to be between 0.2 and 2.5 per cent. Below 0.2 per cent the increase in polarization due to the uranium-malic complex is so small that a small error in reading may make a relatively large error in the final result, while above 2.5 per cent the increase in specific rotation tends to give high recoveries.

The results shown in Table VI illustrate the applicability of the method to natural products. The strawberry juice used as a solvent was highly colored and most of the readings had to be made in 50 or 100 mm polariscope tubes.

TABLE V.—*Determinations on solutions containing known amounts of malic acid.*

Number of determination.	Invert sugar present (approximate).	Malic acid present.	Polarization.			Difference between readings.	Malic acid found.	Recovery.
			Solution 1—with uranium acetate.	Solution 2—without uranium acetate.	Solution 3—with uranium acetate after removing malic acid.			
	Per cent.	Per cent.	° V.	° V.	° V.	° V.	Per cent.	Per cent.
1	10.0	2.98	— 94.0	— 11.9	....	82.1	2.96	99.3
2	....	2.49	— 73.9	....	....	73.9	2.66	106.8
3	5.0	2.49	— 77.6	— 6.6	....	71.0	2.56	102.8
4	5.0	2.49	— 75.0	— 6.6	....	68.4	2.46	98.8
5 [1]	25.0	2.31	— 93.2	— 33.2	— 36.5	60.0	2.16	93.5
6	25.0	1.99	— 85.0	— 29.0	....	56.0	2.02	101.5
7	25.0	1.99	— 82.9	— 32.9	....	50.0	1.80	90.5
8	....	.99	— 26.1	....	....	26.1	.94	94.9
9	5.0	.99	— 32.1	— 5.4	....	26.7	.96	97.0
10	25.0	.99	— 59.2	— 31.9	....	27.3	.98	99.0
11	25.0	.99	— 54.9	— 29.0	....	25.9	.93	93.9
12	5.0	.59	— 22.7	— 6.0	....	16.7	.60	101.7
13	....	.50	— 12.7	....	....	12.7	.46	92.0
14 [1]	5.0	.49	— 18.7	— 5.9	— 6.4	12.8	.46	93.9
15 [1]	10.0	.49	— 26.6	— 12.3	— 13.0	14.3	.51	104.1
16 [1]	15.0	.49	— 31.0	— 17.8	— 18.6	13.2	.48	98.0
17 [1]	25.0	.49	— 45.1	— 31.4	— 32.1	13.7	.49	100.0
18	5.0	.39	— 16.9	— 6.0	....	10.9	.39	100.0
19	2.5	.35	— 12.3	— 2.7	....	9.6	.35	100.0
20 [1]	5.0	.25	— 12.5	— 6.0	— 6.2	6.5	.24	96.0
21 [1]	10.0	.25	— 18.7	— 12.0	— 12.2	6.7	.24	96.0
22 [1]	15.0	.25	— 23.8	— 17.2	— 17.4	6.6	.24	96.0
23 [1]	25.0	.25	— 37.8	— 31.6	— 31.9	6.2	.22	88.0
24	2.5	.20	— 8.1	— 2.7	....	5.4	.19	95.0
25	5.0	.20	— 11.6	— 6.0	....	5.6	.20	100.0
26	....	.20	— 5.2	....	....	5.2	.19	95.0
27	12.5	.20	— 18.7	— 14.2	— 13.7	5.0	.18	90.0
28	25.0	.20	— 34.5	— 30.0	— 29.1	5.4	.19	95.0
29	25.0	.20	— 33.3	— 29.5	— 28.3	5.0	.18	90.0
30	5.0	.19	— 10.1	— 5.1	....	5.0	.18	94.7
31	2.5	.099	— 5.2	— 2.6	....	2.6	.094	94.9
32	5.0	.098	— 8.6	— 6.0	— 6.0	2.5	.094	95.9
33	2.5	.050	— 3.8	— 2.6	....	1.2	.043	86.0

[1] Determinations by C. W. Clark.

TABLE VI.—*Malic acid determinations on unknown solutions.*

Number of determinations.	Solvent.	Malic acid present.	Polarizations.			Difference between readings.	Malic acid found.	Recovery.
			Solution 1—with uranium acetate.	Solution 2—without uranium acetate.	Solution 3—with uranium acetate after removing malic acid.			
		Per cent.	° *V.*	° *V.*	° *V.*		*Per cent.*	*Per cent.*
1...........	Bottled strawberry juice.	0.25	− 8.1	−2.5	....	5.6	0.27	108.
2...........	.....do............	.90	−22.6	−3.8	....	18.8	.90	100.0
3...........	.....do............	.25	− 8.2	−4.3	....	3.9	.19	76.0
4...........	Fresh strawberry juice.	.50	−12.4	−2.6	....	9.8	.47	94.0
5 [1]........	.....do............	.50	−12.0	−1.8	−2.2	10.2	.49	98.0
6 [1]........	.....do............	.37	−10.0	−2.6	−2.4	7.6	.37	100.0
7 [1]........	.....do............	.62	−13.6	−2.1	−3.0	11.5	.55	88.7

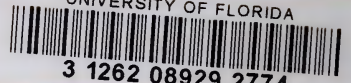[1] Determinations by C. W. Clark.

SUMMARY.

(1) When a neutralized solution of malic acid is treated with uranyl acetate, its rotation is increased approximately 28° V. for each per cent of malic acid in the solution; *d*-tartaric acid is the only other common acid which is affected in this way by uranyl acetate, Hence, in the absence of *d*-tartaric acid, malic acid may be determined quantitatively by treating its solution with uranyl acetate, polarizing, and multiplying the difference between this reading and that of the untreated solution by 0.036. The product equals the percentage of malic acid present.

(2) In the presence of more than 10 per cent of reducing sugars and less than 0.25 per cent of malic acid, the results may be affected by the action of uranyl acetate on the rotation of the sugar. Hence, in this case or when the amounts of sugar or malic acid are unknown, certain simple modifications are necessary.

(3) For this determination, the most favorable limits of concentration of malic acid are between 0.2 to 2.5 per cent. The percentage error seldom amounts to more than 5 per cent of the malic acid present. Twelve complete determinations may easily be made in four hours time, including two hours during which the solutions require no treatment other than frequent shaking.

○